

Cedestis granadensis sp. nov., a new species from Spain (Lepidoptera, Yponomeutidae)

ZDENKO TOKÁR¹, FRIEDMAR GRAF², PETER HUEMER³

1 P. J. Šafárika 11, SK-92700 Šaľa, Slovakia; zdeno.tokar@gmail.com

2 Burtlehn 1, D-02625, Bautzen, Germany; friedmargraf@googlemail.com

3 Tiroler Landesmuseen Betriebsges.m.b.H., Naturwissenschaftliche Sammlungen, Sammlungs- und Forschungszentrum, Krajnc-Str. 1, A-6060 Hall in Tirol, Innsbruck, Austria; p.huemer@tiroler-landesmuseen.at

<https://zoobank.org/1239CF97-1F55-483B-AD43-24AFA7FCE9DA>

Received 18 February 2024; accepted 14 March 2024; published: 4 April 2024

Subject Editor: Bernard Landry.

Abstract. *Cedestis granadensis* sp. nov. is described from specimens taken in two localities in the Spanish province of Granada, in the highlands north of Puebla de Don Fadrique, and the mountains of Sierra de Huétor. The new species belongs to the genus *Cedestis* Zeller, 1839 in the family Yponomeutidae Stephens, 1829. It does not resemble any of the six other known species of this genus in external characters and it also differs significantly from species already barcoded according to the results of a DNA analysis. The immature stages of the new species are unknown. Colour photographs of the adults, the male genitalia, and of the habitat are included with an illustration of the female genitalia.

Introduction

Cedestis Zeller, 1839 is a small genus in the large family Yponomeutidae Stephens, 1829. In Europe, three valid species are known hitherto: *C. subfasciella* (Stephens, 1834) (= *C. farinatella* Zeller, 1839), *C. gysseleniella* (Zeller, 1839), and *C. civitatensis* Nel & Varenne, 2015. Two further species are known from Asia: *C. exiguata* Moriuti, 1977 and *C. leucopterostigmatis* Sohn et al. 2010, and one from tropical Africa: *C. nathani* Agassiz, 2019 (Lewis and Sohn 2015; Agassiz 2019; Lepiforum 2024). Two species from Canada await formal description. There have been several contributions to our understanding of the taxonomy of this group within the family Yponomeutidae, notably Friese (1960) and Gershenson and Ulenberg (1998).

In mid-July 2010, while surveying the Lepidoptera fauna of the Spanish province of Granada, the first author found one female of an unknown species near the Natural Park of Sierra de Huétor at an elevation of about 1400 m. He mistakenly classified this specimen as an unknown species in the Elachistidae family and sent it for determination, along with several other light-coloured *Elachista* species to Laura Kaila, a well-known expert on this family. After evaluating the results of the DNA analysis, Kaila returned that specimen with a note that it did not belong to that family. The first author then examined the genitalia of the female and included it as an unknown species in the family Yponomeutidae.

During a collecting trip in the summer of 2021 from Germany to the south of Spain, the second author decided to occasionally photograph small moths and to take unknown specimens for

identification. Among the material collected at a small plateau at an elevation about 1500 m near the Granada city of Puebla de Don Fadrique was one male specimen, which after examination of its genitalia was assigned to the family Yponomeutidae. DNA barcoding of the specimen subsequently specified its inclusion in the ermine moths, in the genus *Cedestis*.

The third author, while preparing a short manuscript on interesting new records of moths from North Cyprus, while analysing the barcoding data of *Cedestis civitatensis* encountered the problem that the barcoding tree of related species contains a separate cluster of an unknown species, consisting of data of both the above-mentioned specimens from Granada. He informed both discoverers to this situation, which served as a motivating factor for producing the following description of the new species.

Material and methods

Both the examined specimens were taken as adults, having been attracted to light. The genitalia were dissected following the usual procedure for small Lepidoptera (Robinson 1976) except that preparations were temporarily stored in glycerol in small plastic vials. The drawing of the female genitalia was made with Indian ink and a coloured pencil on transparent drawing paper. Photographs of the adults, male genitalia and the type localities were taken using digital cameras Sony Alpha NEX-5R, Sony ILCE-5100 with the lens model E 30 mm F3.5 Macro, Nikon D3100, and Konica Minolta DiMAGE Z5. A Nectaris stereomicroscope with a microshift and a 4× plan achromatic lens were used to photograph the head.

Tissue samples (a single hind leg) from 27 *Cedestis* specimens (14 *C. subfasciella*, 7 *C. gyssele-niella*, 4 *C. civitatensis*, 2 *C. granadensis* sp. nov.) were prepared according to prescribed standards to obtain DNA barcode sequences of a 658 bp segment of the mitochondrial COI gene (cytochrome c oxidase subunit 1). This material was successfully processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using the standard high-throughput protocol described in deWaard *et al.* (2008). In addition, four public DNA barcode sequences (3 *C. exiguata*, 1 *C. nathani*) in the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007; Ratnasingham 2018) were considered for analysis. Further details including complete voucher data and images can be accessed in the public dataset in BOLD DS-CEDEGRAN “New species of *Cedestis* (Yponomeutidae)” (dx.doi.org/10.5883/DS-CEDEGRAN).

All sequences were assigned to Barcode Index Numbers (BIN), algorithm-based operational taxonomic units that usually provide an accurate proxy for valid species. BINs were automatically calculated for records in BOLD that comply with the DNA barcode standard (Ratnasingham and Hebert 2013).

Degrees of intra- and interspecific variation of DNA barcode fragments were calculated using the Kimura two-parameter model on the platform of BOLD systems v. 4.0. (Ratnasingham 2018). A Neighbor-Joining tree was constructed using the Kimura two-parameter model in MEGA7 (Kumar *et al.* 2016).

Molecular analysis

Molecular analysis is based on 31 DNA barcode sequences ranging between 544 and 658 bp. Despite considerable intraspecific variation of up to 2.85% in *C. subfasciella*, DNA barcodes of the available 6 (out of 7) described species group into six strongly divergent clusters, each with different BINs (Fig. 11). The DNA barcoded specimens of *C. granadensis* sp. nov. form a unique BIN: BOLD:AAZ9164 (n = 2) that includes DNA barcodes ID TLMF Lep 32615 and ID MM19987.

Sequences in this BIN are divergent from the closest BIN (BOLD:ADG4688, *Cedestis civitatensis*) (n = 4) by a minimum p-distance of 3.25%. Although this interspecific distance is at the lower level within *Cedestis* (see Table 1), it clearly supports species-level divergence.

Abbreviations

BOLD	Barcode of Life Data System
Gp	Genitalia preparation
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
ZT	Zdenko Tokár

Table 1. Intra- and interspecific p-distances (in %) of *Cedestis* spp. and nearest BIN and species (NN = Near-est Neighbour).

Species	BIN	n	av. dist.	max. dist.	dist. NN	BIN NN	NN species
<i>Cedestis civitatensis</i>	BOLD:ADG4688	4	0	0	3.25	BOLD:AAZ9164	<i>Cedestis granadensis</i>
<i>Cedestis exiguata</i>	BOLD:AAF2140	3	0.2	0.31	4.08	BOLD:AAC5311	<i>Zelleria retiniella</i>
<i>Cedestis granadensis</i>	BOLD:AAZ9164	2	0	0	3.25	BOLD:ADG4688	<i>Cedestis civitatensis</i>
<i>Cedestis gysseleniella</i>	BOLD:AAE4109	24	0.46	1.57	7.41	BOLD:AAE9370	<i>Cedestis subfasciella</i>
<i>Cedestis nathani</i>	BOLD:ABW6374	4	0	0	6.57	BOLD:ABW6332	unid. Yponomeutidae
<i>Cedestis subfasciella</i>	BOLD:AAE9370	41	1.07	2.85	6.63	BOLD:AAC5311	<i>Zelleria retiniella</i>

Taxonomy

Cedestis granadensis Tokár, Graf & Huemer, sp. nov.

<https://zoobank.org/429F60EC-A55E-4A68-9D8A-7E8E60329DC9>

Material. Holotype: ♂, Spain, Andalusia, Prov. Granada, Highlands north of Puebla de Don Fadrique, 1508 m, 18.vii.2021, 38°00'32.4"N, 2°28'50.4"W. Original labels: “Spanien, Andalusien, Granada, Hochland nördlich Puebla de Don Fadrique, 1508 m, 38.009006, -2.480653, 18.vii.2021 am Licht”, “DNA Barcode TLMF Lep 32615” (green label), “HOLOTYPE *Cedestis granadensis* Tokár, Graf & Huemer” (red label), leg. F. Graf, coll. TLMF.

Paratype: ♀, Spain, Andalusia, Prov. Granada, Sierra Puerto de la Mora de Huétor, 1400 m, 11.vii.2010, 37°16'02.0"N, 3°25'40.2"W, Gp. ZT 10991, DNA sample Lepid Phyl 19987, leg. & coll. Z. Tokár. “PARATYPE *Cedestis granadensis* Tokár, Graf & Huemer” (red label).

Description. Adult (Figs 1–4). Male and female almost identical (forewing markings in female are slightly less pronounced than in male). Wingspan 9.5–10.0 mm. Head: frons white, vertex with whitish to light yellowish brown scale tufts, labial palpi small, straight, segment 3 slightly longer than segment 2, whitish. Scape whitish, flagellum annulate dark brown and whitish, ¾ length of wing. Thorax and tegula whitish. Forewing ground colour white to creamy white, slightly shining. Markings with two-tone scales, light yellowish brown with dark brown tips. An irregular scattering of darker scales at 1/3 of forewing in the fold, together with faint dark dusting towards both margins give a hint of an indistinct bent fascia. Further scatterings of darker scales at the base of costa, and in dorsum closer towards tornus. Individual scattered darker scales especially towards the apex. Cilia white with ciliary line and other scattered darker scales. Hindwing and cilia light grey-brown. Underside of both wings grey-brown, darker on the forewing, with darker veins.



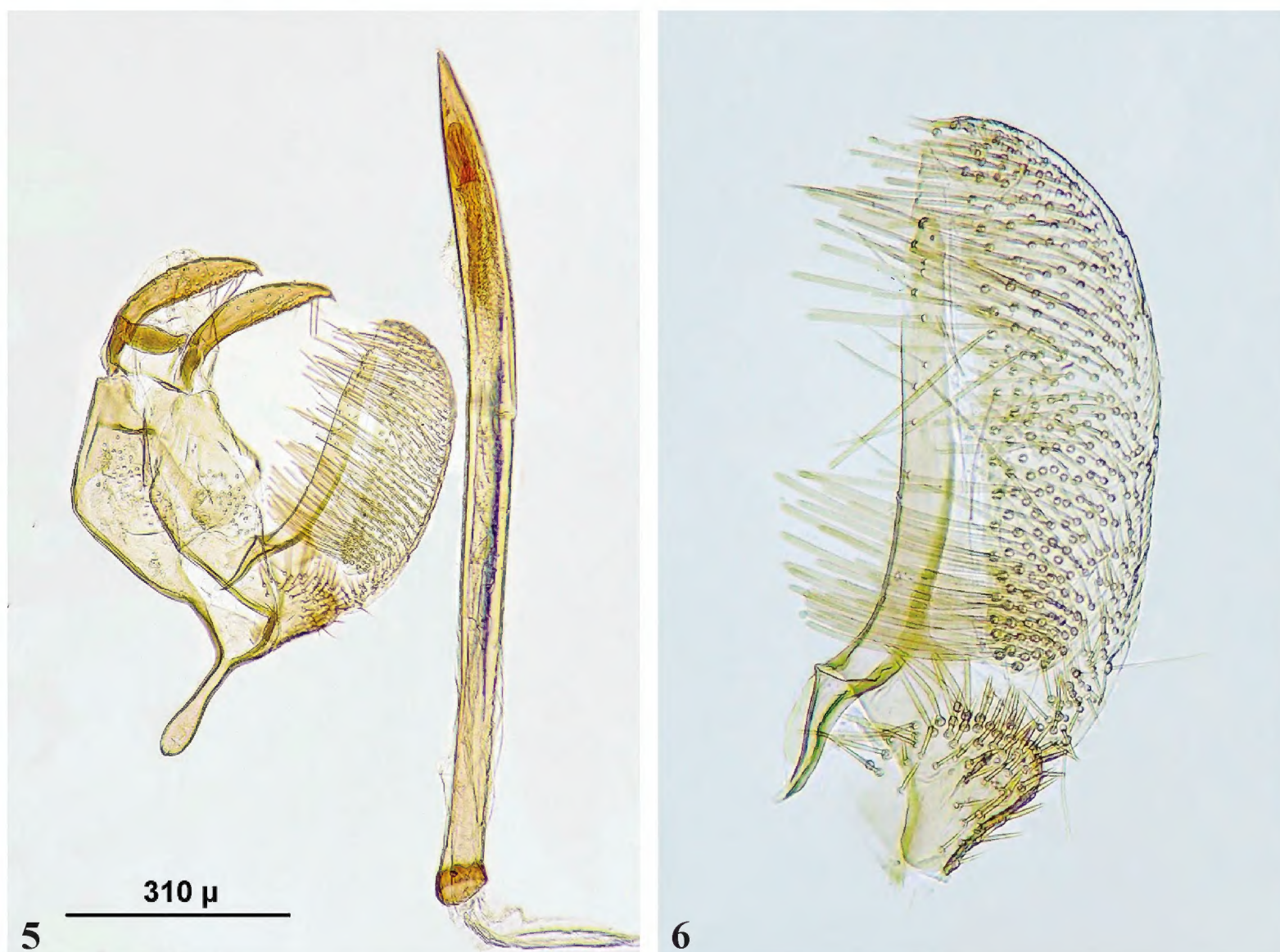
Figures 1, 2. *Cedestis granadensis* sp. nov., male, holotype, highlands north of Puebla de Don Fadrique, 18.vii.2021, **1.** The resting adult; **2.** Dorsal view, wingspan 10.0 mm. Photographs F. Graf.

Male genitalia (Figs 5, 6). Uncus broad with slightly protruding and rounded lobes; socii long, wider at 1/2–2/3 length, pointed at apex; gnathos small, inconspicuous; valva semi-ovate, costal margin almost straight, sacculus semi-oval; phallus long, slightly curved at 2/3 length, pointed at apex, vesica with triangular cornutus on spatulate structure; saccus relatively short, clavate.

Female genitalia (Figs 7, 8). Papillae anales elongate, sclerotized, covered with long and short setae. Apophyses posteriores slightly longer than papillae anales. Ventral and dorsal branches of apophyses anteriores shorter than apophyses posteriores; ventral branches ending in small elongated lamella postvaginalis in eighth segment. In posterior part of lamella, oval ostium bursae covered



Figures 3, 4. *Cdestis granadensis* sp. nov., female, paratype, Sierra de Huétor Mts., 11.vii.2010, **3.** Dorsal view; **4.** Head, ventral view. Scale bars: 1.0 mm. Photographs F. Slamka.



Figures 5, 6. Male genitalia of *Cdestis granadensis* sp. nov., holotype; **5.** Ventral view with right valva; **6.** Left valva. Photographs F. Graf.

with microtrichia between pair of setose humps. Antrum sclerotized, cup-shaped. Ductus bursae moderately long, folded in anterior part, finely papillate anteriorly from three-quarters, gradually expanding into oval corpus bursae with continued papillation posteriorly. Signum large, elliptic with transverse expansion, covered with numerous triangular teeth of various sizes.



Figures 7, 8. Female genitalia of *Cedestis granadensis* sp. nov., paratype, Gp. Z. Tokár ♀ 10991. Scale bars: 1.0 mm. Drawing Z. Tokár.

Diagnosis. The male and female genitalia of *Cedestis granadensis* sp. nov. closely resemble those of *C. civitatensis*. The male genitalia of the new species differ from the latter mainly in having the broad uncus with slightly protruding and rounded lobes, whilst in the latter the uncus is truncated. In the female genitalia, the new species can be distinguished from *C. civitatensis* by the longer apophyses posteriores and anteriores and by the form and structure of the lamella postvaginalis, the ostium bursae covered with microtrichia between the pair of small setose humps in the posterior part of the lamella, whilst in *C. civitatensis* the lamella postvaginalis has another form and structure. The new species is furthermore significantly distinguished from all other known *Cedestis* species by the white ground colour and sparse pattern on the forewing as well as by large distances in the DNA barcode.

Distribution. So far only known from the two localities in the Spanish province of Granada: highlands north of Puebla de Don Fadrique, and in mountains of the Sierra de Huétor. Both sites are at elevations of approximately 1400–1500 m.

Biology. The biology is unknown. Both adults of the new species were on the wing in July. The habitats were a small rocky-steppe plateau, surrounded by groups of pines (near Puebla de Don Fadrique, Fig. 9) or clearings of sparse pine forest on dolomitic sands (Sierra de Huétor, Fig. 10).



Figure 9. Locality of *Cedestis granadensis* sp. nov. north of Puebla de Don Fadrique. Photograph F. Graf.



Figure 10. Locality of *Cedestis granadensis* sp. nov. in Sierra de Huétor Mts. Photograph Z. Tokár.

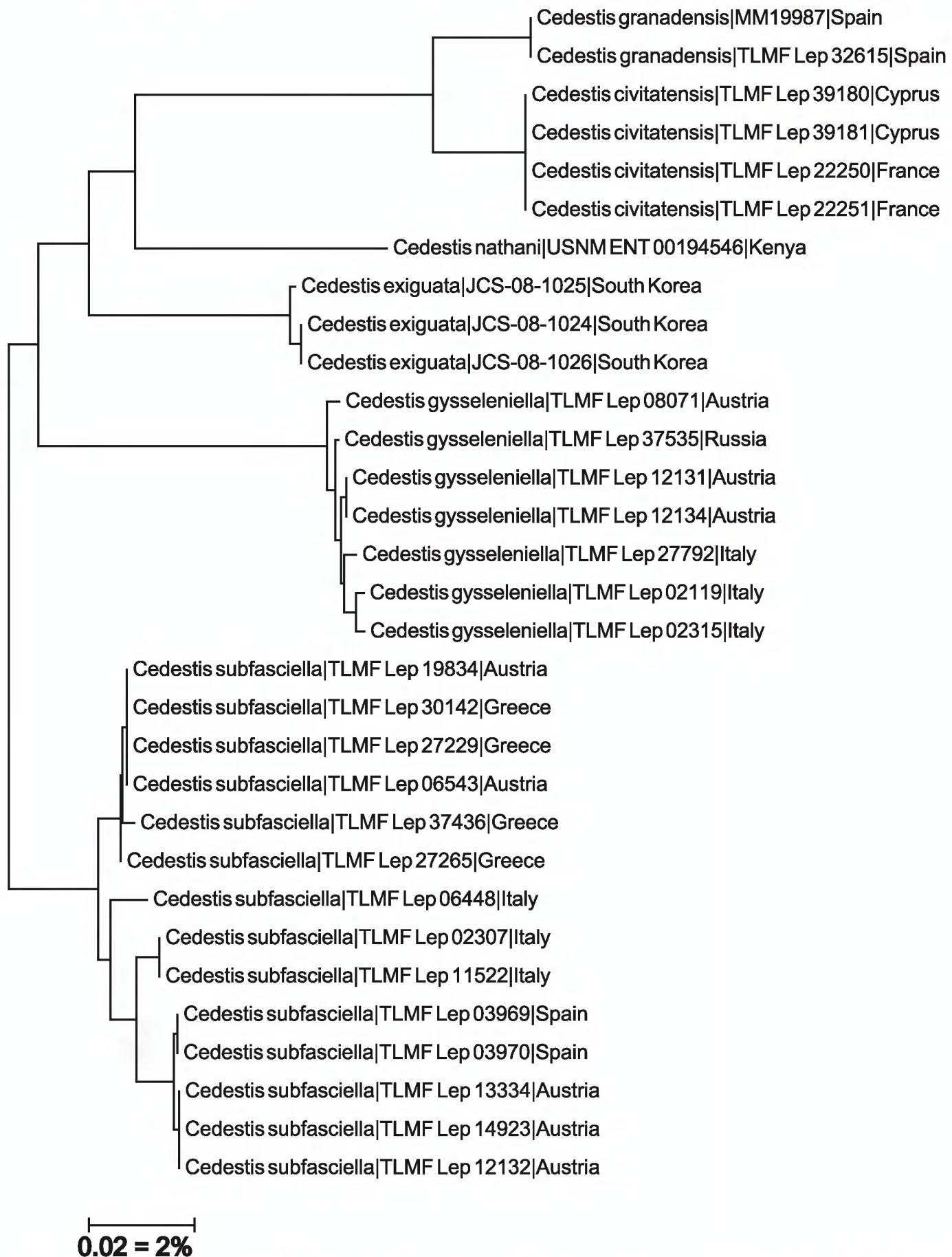


Figure 11. Unrooted Neighbor-joining tree of *Cedestis granadensis* sp. nov. and selected closely related species based on COI barcode sequences with BOLD IDs and countries. Scale bar represents 2% K2P genetic divergence between sequences.

The life history of the two European *Cedestis* species with a Palearctic distribution (*C. gysseleniella*, *C. farinatella*) is very well known. Larvae of these species mine exclusively in needles of Pinaceae (*Pinus sylvestris* L., *P. mugho* Turra, *Abies alba* Mill.) and they live from the autumn and after hibernation to the spring (Fries 1960). The African species (*C. nathani*) has been reared

from *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae) (Agassiz 2019). Host-plants of Asian *Cedestis* species or the recently described species *C. civitatensis* are unknown.

We can assume that the larva of our new species also develops in a similar way like the larvae of the two European species mentioned above, in needles of some species of Pinaceae occurring at the type localities (*Abies pinsapo* Boiss., *Pinus halepensis* Mill., *P. nigra* subsp. *salzmannii* (Dunal) Franco, *P. pinaster* Aiton, *P. sylvestris* var. *nevadensis* D.H. Christ) in the Sierra de Huétor Natural Park (Junta de Andalucía 2020).

Etymology. The specific name *granadensis* is derived from Granada, the Spanish province in the autonomous region of Andalusia, where both specimens of the new species were discovered.

Discussion. The documentation of the biodiversity of Lepidoptera in Mediterranean countries seems far from complete despite extensive recording efforts. Particularly, increasingly comprehensive genetic surveys implemented in the last 15 years have revealed significant potential for cryptic diversity both in the analysis of larger taxonomic units (Huemer et al. 2020; Lopez-Vaamonde et al. 2021) and in larger faunistic projects (Huemer and Mutanen 2022; Huemer and Wieser 2023). In extreme cases, the extent of overlooked taxa comprises up to a third of the species or even more (Huemer and Hebert 2011; Mutanen et al. 2013; Huemer and Karsholt 2018).

In this context, the discovery of a new species of the genus *Cedestis* in Spain is not entirely surprising, especially considering that a congeneric species, *C. civitatensis*, was recently discovered in the already well-explored southern France (Nel and Varenne 2015). However, it is striking that the new discovery appears phenotypically so distinctive that confusion should actually be ruled out, suggesting a certain rarity. Whether it is an endemic species of southern Spain is doubtful, given the widespread distribution of potential larval host plants of the genus *Pinus*. Additionally, another further occurrence of the sister species in southern France has been discovered in Cyprus, indicating a much more widespread distribution pattern (Huemer and Özden 2024).

While the phylogeny of the Yponomeutoidea has been convincingly clarified at the suprageneric level by Sohn et al. (2013), generic revisions are evidently still needed. Although the close relationship between *C. granadensis* sp. nov. and *C. civitatensis* is well supported both morphologically and molecularly, the generic placement of several species of the related genus *Zelleria* Stainton, 1849 appears doubtful and in need of revision based on genetic data.

Acknowledgements

Our thanks are due to Lauri Kaila (Finnish Museum of Natural History, Helsinki, Finland) and Marko Mutanen (University of Oulu, Finland) for providing the DNA barcoding of one specimen. We are further grateful to Paul D.N. Hebert and the entire team at the Canadian Centre for DNA Barcoding (Guelph, Canada), whose sequencing work was enabled through funding from Genome Canada through Ontario Genomics, and to the Ontario Ministry of Research and Innovation and Natural Sciences and Engineering Council of Canada for their support of the BOLD informatics platform. František Slamka (Bratislava, Slovakia) is thanked for photographs of the female adult and its head. We are grateful to Robert J. Heckford (Plympton, UK) and Stella D. Beavan (Zeal Monachorum, UK) for checking and correcting the English, and helpful comments. Martin Corley (Faringdon, UK), David Agassiz (Natural History Museum, London, UK) as reviewers, and Bernard Landry (Natural History Museum of Geneva, Switzerland) as an editor, improved the article with valuable suggestions.

References

- Agassiz DJL (2019) The Yponomeutidae of the Afrotropical region (Lepidoptera: Yponomeutoidea). *Zootaxa* 4600: 1–69. <https://doi.org/10.11646/zootaxa.4600.1.1>
- deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA barcodes: analytical protocols. In: Martin CC (Ed.) *Methods in Molecular Biology: Environmental Genomics*. Humana Press Inc., Totowa, USA, 275–293. https://doi.org/10.1007/978-1-59745-548-0_15
- Friese G (1960) Revision der paläarktischen Yponomeutidae unter besonderer Berücksichtigung der Genitalien (Lepidoptera). *Beiträge zur Entomologie* 10(1/2): 1–131.
- Gershenson ZS, Ulenberg SA (1998) The Yponomeutinae (Lepidoptera) of the World exclusive of the Americas. Koninklijke Nederlandse Akademie van Wetenschappen. *Verhandelingen Afdeling Natuurkunde* 2(99): 1–202.
- Huemer P, Hebert PDN (2011) Cryptic diversity and phylogeography of high alpine *Sattleria* — a case study combining DNA barcodes and morphology (Lepidoptera: Gelechiidae). *Zootaxa* 2981: 1–22. <https://doi.org/10.11646/zootaxa.2981.1.1>
- Huemer P, Karsholt O (2018) Revision of the genus *Megacraspedus* Zeller, 1839, a challenging taxonomic tightrope of species delimitation (Lepidoptera, Gelechiidae). *ZooKeys* 800: 1–278. <https://doi.org/10.3897/zookeys.800.26292>
- Huemer P, Karsholt O, Aarvik L, Berggren K, Bidzilya O, Junnilainen J, Landry J-F, Mutanen M, Nupponen K, Segerer A, Šumpich J, Wieser C, Wiesmair B, Hebert PDN (2020) DNA barcode library for European Gelechiidae (Lepidoptera) suggests greatly underestimated species diversity. *ZooKeys* 921: 141–157. <https://doi.org/10.3897/zookeys.921.49199>
- Huemer P, Mutanen M (2022) An Incomplete European Barcode Library Has a Strong Impact on the Identification Success of Lepidoptera from Greece. *Diversity* 14: 118. <https://doi.org/10.3390/d14020118>
- Huemer P, Özden Ö (2024) Molecular identification of newly recorded Lepidoptera for Cyprus and Europe. *SHILAP Revista de lepidopterología* 52 (submitted).
- Huemer P, Wieser C (2023) DNA Barcode Library of Megadiverse Lepidoptera in an Alpine Nature Park (Italy) Reveals Unexpected Species Diversity. *Diversity* 15: 214. <https://doi.org/10.3390/d15020214>
- Junta de Andalucía, Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible (2020) 1º Borrador del II Plan de Desarrollo Sostenible, Parque Natural Sierra de Huétor, Versión febrero 2020, 158 pp.
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lepiforum eV [Ed.] (2024) *Cedestis* in Europa. In: Lepiforum eV (Ed.) (2008–2024) *Bestimmungshilfe für die in Europa nachgewiesenen Schmetterlingsarten*. [Available from] <https://www.lepiforum.org> [Accessed 23 January 2024]
- Lewis JA, Sohn J-Ch (2015) Lepidoptera: Yponomeutoidea I (Argyresthiidae, Attevidae, Praydidae, Scythropiidae, and Yponomeutidae). In: Landry B (Ed.) *World catalogue of insects* 12, Brill, Leiden and Boston, 253 pp. <https://doi.org/10.1163/9789004264267>
- Lopez-Vaamonde C, Kirichenko N, Cama A, Doorenweerd C, Godfray HCF, Guiguet A, Gomboc S, Huemer P, Landry J-F, Laštůvka A, Laštůvka Z, Lee KM, Lees DC, Mutanen M, Nieukerken EJ van, Segerer AH, Triberti P, Wieser C, Rougerie R (2021) Evaluating DNA barcoding for species identification and discovery in European gracillariid moths. *Frontiers in Ecology and Evolution* 9: 626752. <https://doi.org/10.3389/fevo.2021.626752>
- Mutanen M, Kaila L, Tabell J (2013) Wide-ranging barcoding aids discovery of one-third increase of species richness in presumably well-investigated moths. *Scientific Reports* 3: 2901. <https://doi.org/10.1038/srep02901>

- Nel J, Varenne T (2015) Descriptions de *Phyllonorycter acericorsica* sp. n. et de *Cedestis civitatensis* sp. n.; *Bactra simpliciana* Chrétien, 1915, espèce nouvelle pour l'Europe (Lepidoptera, Gracillariidae, Yponomeutidae, Tortricidae). *Revue de l'Association Roussillonnaise d'Entomologie* 24(4): 162–168.
- Ratnasingham S (2018) BOLD Barcode of Life Data System, version 4. <http://www.boldsystems.org> [Accessed 16 January 2024]
- Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355–364. <https://doi.org/10.1111/j.1471-8286.2007.01678.x>
- Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLOS ONE* 8: e66213. <https://doi.org/10.1371/journal.pone.0066213>
- Robinson GS (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. *Entomologist's Gazette* 27: 127–132.
- Sohn J-Ch, Regier JC, Mitter C, Davis D, Landry J-F, Zwick A, Cummings MP (2013) A Molecular Phylogeny for Yponomeutoidea (Insecta, Lepidoptera, Ditrysia) and Its Implications for Classification, Biogeography and the Evolution of Host Plant Use. *PLOS ONE* 8(1): 1–23. e55066. <https://doi.org/10.1371/journal.pone.0055066>
- Sohn J-Ch, Wu Ch-Sh, Han H-L (2010) Three new species of Yponomeutinae (Lepidoptera: Yponomeutidae) from China with faunistic supplements and an updated list of the Chinese species of the subfamily. *Journal of Natural History* 44(45/46): 2803–2816. <https://doi.org/10.1080/00222933.2010.503942>
- Stephens JF (1834) *Illustrations of British Entomology; or, a Synopsis of Indigenous Insects: containing their generic and specific distinctions; with an account of their metamorphoses, times of appearance, localities, food, and economy, as far as practicable.* Haustellata 4. Baldwin & Cradock, London, England, 433 pp.
- Zeller PC (1839) Versuch einer naturgemäßen Eintheilung der Schaben. *Isis von Oken* 32(3): 167–220.